Intestinal fatty acid binding protein and microsomal triglyceride transfer protein polymorphisms in French-Canadian youth

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Abstract **Growing evidence suggests an association between lipid abnormalities and fatty acid binding protein (FABP) and microsomal triglyceride transfer protein (MTP) gene variants. Our objectives were to determine whether Ala54Thr FABP2 and G−493T MTP polymorphisms are associated with increased risks of insulin resistance syndrome (IRS) in youth and/or modify the expression of accompanying dyslipidemia. Our study of 1,742 French-Canadians aged 9, 13, and 16 years did not provide evidence of a potential predisposition to IRS related to either FABP2 or MTP genotypes. However, we observed a heterogeneity of the FABP2 effect by IRS status on total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and apolipoprotein B (apoB) concentrations (*P* for interaction = 0.045, 0.018, and 0.017, respectively). Among the metabolic components of IRS, only triglyceride (TG) displayed an interaction with FABP2 polymorphism: compared with Thr/Ala and Ala/Ala, the Thr/Thr genotype was associated with a steeper increase in TC, LDL-C, and apoB parallel to TG concentrations (*P* < 0.001). IRS did not modify the associations between the MTP polymorphism and any of the biochemical parameters. Our study suggests that the effects of FABP2 allelic variations on lipid traits are context dependent, indicating that this variant may play an important role in cardiovascular pathogenesis in the presence of IRS or hypertriglyceridemia.**—Stan, S., M. Lambert, E. Delvin, G. Paradis, J. O'Loughlin, J. A. Hanley, and E. Levy. **Intestinal fatty acid binding protein and microsomal triglyceride transfer protein polymorphisms in French-Canadian youth.** ***J. Lipid Res.*** **2005.** 46: **320–327.**

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During the past few years, substantial progress has been made toward understanding intestinal fat absorption. It is now well recognized that the formation of triglyceride (TG)-rich lipoproteins within the enterocyte is a multistep process that includes the uptake of lipolytic products and their translocation to the endoplasmic reticulum by fatty acid binding proteins (FABPs) for the reesterification of lipids, the synthesis and posttranslational modification of various apolipoproteins, and ultimately the assembly of chylomicrons (1, 2). Studies of genetic fat transport disorders have afforded new insight into the key functions of crucial intracellular proteins, such as apolipoprotein B (apoB) (3, 4), microsomal triglyceride transfer protein (MTP) (5), and Sar1 GTPase (6–8), the defects of which lead to hypobetalipoproteinemia, abetalipoproteinemia, and chylomicron retention disease, respectively (9). Conversely, intestinal TG-rich lipoprotein overproduction contributes to the dyslipidemia associated with insulin resistance and diabetes (10–12). The abundant formation of TG-rich lipoproteins can be explained in part by a greater stability of intracellular apoB-48, enhanced intestinal enterocyte de novo lipogenesis, and upregulation of intestinal-FABP (I-FABP) and MTP. Moreover, some studies have shown that variations in gene coding for proteins involved in intestinal fat absorption might influence this process. In particular, the G-to-A substitution at codon 54 of the FABP2 gene, which results in an alanine-to-threonine substitution at amino acid 54 (Ala54Thr) of I-FABP, has been reported to be associated with increased intestinal fat absorption (13, 14), FA oxidation (15), insulin resistance,

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Abbreviations: apoB, apolipoprotein B; BMI, body mass index; BP, blood pressure; dNTP, deoxynucleoside triphosphate; FABP, fatty acid binding protein; HDL-C, high density lipoprotein-cholesterol; I-FABP, intestinal fatty acid binding protein; IFG, impaired fasting glucose; IRS, insulin resistance syndrome; LDL-C, low density lipoprotein-cholesterol; MTP, microsomal triglyceride transfer protein; TC, total cholesterol; TG, triglyceride.

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and diabetes (15). However, not all studies concur (16–18). In fact, the association between the FABP2 gene variants and lipid disorders appears to be much more complex than hypothesized, because the same I-FABP mutation had no similar impact on the composition of plasma lipids, the basal metabolic rate, or insulin, glucose, and lipid levels in different populations (19). Therefore, this issue requires more careful investigation, the findings of which may contribute toward better understanding of the specific role of I-FABP variants in exogenous fat transport and postprandial lipemic response. The current work also assessed the influence of the G-493T MTP gene polymorphism, which has also shown an impact on total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and LDL-apoB levels (20, 21).

Therefore, the present investigation was undertaken to examine whether the FABP2 and MTP gene variants are associated with an increased risk of the insulin resistance syndrome (IRS) or whether they modify the expression of the dyslipidemia associated with IRS in a pediatric French-Canadian population. The French-Canadian population, which is primarily and historically located in the province of Quebec, has the highest prevalence worldwide of lipoprotein lipase deficiency. It includes a large pool of individuals at risk for atherosclerosis and other lipid-related diseases (22, 23). These abnormalities are presumably related to a founder effect among the 8,000 ancestors of present-day French-Canadians, who have had relatively little cross-breeding with individuals from other national origin groups (24).

EXPERIMENTAL PROCEDURES

Population study

The design and methods of the 1999 Quebec Child and Adolescent Health and Social Survey, a school-based survey of youth aged 9, 13, and 16 years, have been reported in detail (25, 26). A total of 2,244 fasting plasma and DNA samples were available (25). We restricted the current analysis to 1,742 children and adolescents of French-Canadian origin to reduce the confounding of genetic analyses by population stratification. The study was approved by the Ethics Review Board of Ste-Justine Hospital. Written informed consent was obtained from parents/guardians, and written informed assent was obtained from study participants.

Anthropometry, blood pressure, and lipids

Height, weight, and blood pressure (BP) were measured according to standardized protocols (26). Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared. Values of percentile cutoff points used to identify subjects with metabolic risk factors were estimated from the study distributions. Cutoff points were age and sex specific, and BP cutoff points were also height specific, according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (27). Subjects with BMI $\geq$ 85th percentile values were categorized as overweight. High TG, insulin, and systolic BP were defined as values $\geq$ 75th percentile, and low high density lipoprotein-cholesterol (HDL-C) was defined as values $\leq$ 25th percentile. Impaired fasting glucose (IFG) was defined as concentrations $\geq$6.1 and $<$7.0 mmol/l. No study participant had fasting plasma glucose $\geq$ 7.0 mmol/l. There is no internationally accepted definition of childhood IRS; categorization of subjects as having IRS in our analyses required the presence of hyperinsulinemia and at least two of five risk factors: overweight, high systolic BP, high TG, low HDL-C, and IFG. Current smokers were defined as those who responded positively to the question: "During the past 30 days, did you smoke cigarettes, even just a few puffs?" This question was not asked of 9 year olds. Only 2.1% of this age group responded positively to the question: "Have you ever smoked a whole cigarette?" Therefore, all 9-year-old individuals were classified as nonsmokers. Subjects who consumed alcohol regularly were defined as those who responded "about once a week" to the question: "During the last 12 months, how often did you drink alcohol? (just to taste, less than once a month, about once a month, about once a week)."

Biochemical analyses

Blood samples were collected in the morning, after an overnight fast. Plasma TC, HDL-C, TG, and glucose concentrations were determined on a Beckman Synchron Cx7 instrument (25, 26). ApoA-I and apoB were measured by nephelometry (Array Protein System; Beckman). The Friedewald equation was used to calculate LDL-C. Plasma insulin concentration was determined with the ultrasensitive Access® immunoassay system (Beckman Coulter, Inc.), which has no cross-reactivity with proinsulin or C-peptide. Plasma FFA concentrations were quantified by an enzymatic colorimetric method (Wako Chemicals).

Polymorphism detection

Genomic DNA was prepared from white blood cells using the Puregene® DNA Isolation kit (Gentra Systems, Inc.). A 228 bp DNA fragment containing the G-to-A substitution at codon 54 was amplified using the following primers: 5′ CAC TTC CTA TGG GAT TTG ACT 3′ and 5′ TAC CCT GAG TTC AGT TCC GTC 3′. PCR was carried out in a 25 μl reaction volume containing 2 μmol/l of each primer, 200 μmol/l deoxynucleoside triphosphate (dNTP; Pharmacia), 1 mmol/l $MgCl_2$, 2 units of Taq DNA polymerase (Gibco-BRL), 67 mmol/l Tris-HCl, pH 8.3, and 100 ng of genomic DNA. After an initial 5 min denaturation at 95°C, amplifications were carried out for a total of 30 cycles of 94°C for 45 s, 55°C for 60 s, 72°C for 45 s, and completed with a final 7 min extension at 72°C. To detect the Ala54Thr polymorphism, PCR products were blotted on nylon membranes, hybridized at 43°C with digoxigenin-labeled probes corresponding to the Ala allele (5′ GAA TCA AGC GCT TTT CGA A 3′) and the Thr allele (5′ GAA TCA AGC ACT TTT CGA A 3′), washed at 39°C and 42°C, respectively, and autoradiographed. For quality control purposes, genotyping of a systematic random sample of 1 in 10 specimens was repeated using digestion of the PCR products with the *Hha*I restriction enzyme (Gibco-BRL) to detect gene variants, as described by Baier et al. (15). For all samples, reading of the genotype was independently carried out by two individuals.

The DNA fragment used for the detection of the G-493T polymorphism by allele-specific hybridization was obtained by PCR. The amplification was preceded by denaturation at 94°C for 5 min and performed for 30 cycles of 94°C for 60 s, 57°C for 60 s, and 72°C for 60 s in a buffer containing 2 μmol/l of each primer (5′ AGT TTC ACA CAT AAG GAC AAT CAT CTA 3′ and 5′ GTA GTA AGG ATT CTC AAA CTC TGC 3′), 200 μmol/l dNTP, 0.8 mmol/l $MgCl_2$, 1.5 units of Taq DNA polymerase, 67 mmol/l Tris-HCl, pH 8.3, and 100 ng of genomic DNA. The probes used for the allele-specific hybridization were MTP-G allele (5′ TGA TTG GTG GTG GTA TGA A 3′) and MTP-T allele (5′ GTG ATT GGT TGT GGT ATG A 3′). The temperatures corresponding to the hybridization and the washdown of the wild (G) and mutated

TABLE 1. Characteristics of study participants according to IRS status

Variable	Total (n = 1,742)	IRS[a]		
		No (n = 1,546)	Yes (n = 196)	P^b
9 year olds, % (n)	31.9 (555)	32.4 (501)	27.6 (54)	0.387
13 year olds, % (n)	31.0 (540)	30.8 (476)	32.7 (64)	
16 year olds, % (n)	37.1 (647)	36.8 (569)	39.8 (78)	
Sex: male, % (n)	50.3 (876)	50.5 (780)	49.0 (96)	0.698
Smoking (yes), % (n)	19.6 (342)	19.7 (304)	19.4 (38)	0.927
Alcohol intake (yes), % (n)	8.7 (152)	9.2 (142)	5.1 (10)	0.561
BMI[c] (kg/m^2)	20.2 ± 4.4	19.4 ± 3.5	26.4 ± 5.6	< 0.001
Systolic BP (mmHg)	111.8 ± 13.7	110.5 ± 13.0	121.9 ± 15.0	< 0.001
Diastolic BP (mmHg)	59.3 ± 7.1	58.8 ± 6.9	63.4 ± 7.4	< 0.001
TC (mmol/l)	3.99 ± 0.75	3.97 ± 0.75	4.16 ± 0.80	0.001
LDL-C (mmol/l)	2.30 ± 0.64	2.28 ± 0.63	2.46 ± 0.68	< 0.001
ApoB (g/l)	0.66 ± 0.18	0.65 ± 0.17	0.74 ± 0.19	< 0.001
HDL-C (mmol/l)	1.30 ± 0.25	1.32 ± 0.25	1.10 ± 0.19	< 0.001
ApoA-I (g/l)	1.19 ± 0.17	1.20 ± 0.17	1.11 ± 0.16	< 0.001
TG[c] (mmol/l)	0.86 ± 0.42	0.81 ± 0.34	1.32 ± 0.65	< 0.001
FFA[c] (mmol/l)	0.44 ± 0.21	0.43 ± 0.21	0.45 ± 0.19	0.050
Glucose (mmol/l)	5.16 ± 0.38	5.14 ± 0.37	5.33 ± 0.41	< 0.001
Insulin[c] (pmol/l)	43.5 ± 30.1	37.5 ± 18.0	90.6 ± 55.1	< 0.001

Data are expressed as percentage (frequency) or mean (SD). ApoB, apolipoprotein B; BMI, body mass index; BP, blood pressure; HDL-C, high density lipoprotein-cholesterol; IRS, insulin resistance syndrome; LDL-C, low density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride.

[a] IRS is defined as the presence of hyperinsulinemia (⩾75th percentile) in combination with two or more of the following: overweight (BMI of ⩾85th percentile), high TG (⩾75th percentile), low HDL-C (⩽25th percentile), high systolic BP (⩾75th percentile), and high glucose (⩾6.1 mmol/l).

[b] *P* value for comparisons between groups (IRS− and IRS+).

[c] Untransformed data are presented; $\log_e$-transformed values were used for statistical comparisons.

(T) probe were 44, 45, and 44°C, respectively. For quality control purposes, genotyping of a systematic random sample of 1 in 10 specimens was repeated using a method adapted from Karpe et al. (28). A 109 bp DNA fragment was obtained by a polymerase chain reaction performed in 25 μl containing 1 μmol/l of each primer (5′ AGT TTC ACA CAT AAG GAC AAT CAT CTA 3′ and 5′ GGA TTT AAA TTT AAA CTG TTA ATT CAT CAT ATC AC 3′), 200 μmol/l dNTP, 3.5 mmol/l $MgCl_2$, 2 units of Taq DNA polymerase, 67 mmol/l Tris-HCl, pH 8.3, and 100 ng of genomic DNA under the following conditions: an initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, 57°C for 60 s, 72°C for 2 min, and a final elongation of 7 min at 72°C. The 109 bp PCR product was digested with *Hph*I New England Biolabs (NEB); the antisense primer used for the PCR introduced a restriction site on the G allele only. For all samples, reading of the genotype was independently carried out by two individuals.

TABLE 2. Association between IRS and FABP2 and MTP genotypes: distribution of FABP2 and MTP alleles and genotypes according to IRS status

Variable	IRS−	IRS+	P^a
FABP2 allele, % (n)			
Ala	73.9 (2284)	75.8 (297)	0.419
Thr	26.1 (808)	24.2 (95)	
FABP2 genotype, % (n)			
Ala/Ala	55.1 (851)	57.1 (112)	0.654
Ala/Thr	37.6 (582)	37.2 (73)	
Thr/Thr	7.3 (113)	5.6 (11)	
MTP allele, % (n)			
G	73.6 (2275)	74.7 (293)	0.621
T	26.4 (817)	25.3 (99)	
MTP genotype, % (n)			
G/G	53.8 (831)	53.6 (105)	0.357
G/T	39.7 (613)	42.4 (83)	
T/T	6.6 (102)	4.1 (8)	

IRS− indicates subjects who did not meet the diagnostic criteria for IRS; IRS+ indicates subjects who met the criteria for IRS. FABP, fatty acid binding protein; MTP, microsomal triglyceride transfer protein.

[a] *P* value for comparisons between groups (IRS− and IRS+).

Statistical analyses

Statistical analyses were performed with SAS statistical software (SAS Institute, Inc.). For each gene locus examined, allele frequencies were estimated by the gene-counting method (29). A Chi-square test was used to determine whether genotypes at each gene locus were in Hardy-Weinberg equilibrium. Subjects were categorized according to their IRS status (yes/no). Between-group allele and genotype frequency distributions were compared by a Chi-square test. To take the design effect into account, mixed models were used for all analyses of variance and regressions, with genetic markers and other independent variables treated as fixed effects and with clustering between subjects in the same

TABLE 3. Association between IRS and FABP2 and MTP genotypes: adjusted odds ratios for the presence of IRS according to FABP2 and MTP genotypes

Variable	Odds Ratio	*P*
FABP2 genotype		
Ala/Ala (n = 963)	1.00	
Ala/Thr (n = 655)	0.98	0.871
Thr/Thr (n = 124)	0.73	0.326
MTP genotype		
G/G (n = 936)	1.00	
G/T (n = 696)	1.05	0.731
T/T (n = 110)	0.57	0.126

Odds ratios are shown for the presence of IRS in subjects with FABP2 Ala/Thr and Thr/Thr genotypes compared with subjects with FABP2 Ala/Ala genotype and in subjects with MTP G/T and T/T genotypes compared with subjects with MTP G/G genotype. Models were adjusted for age, sex, cigarette use, and alcohol intake.

school treated as a random effect. We used mixed logistic regression to examine the association between IRS status and the FABP2 and MTP genotypes. We used mixed ANOVA and mixed linear regression to study the associations between genotypes and metabolic variables. Scheffe's contrasts were used for post-hoc pair comparisons. Insulin, TG, FFA, and BMI values were $\log_e$ transformed for statistical analyses to improve the normality of their distributions. Because we pooled age and sex groups, age- and sex-specific *Z* scores for BMI, insulin, glucose, TG, and HDL-C were used in linear regression analyses. To standardize a value (i.e., compute its *Z* score), we subtracted the mean of the corresponding study distribution and divided by the SD.

RESULTS

The clinical and biochemical characteristics of participants are shown in **Table 1**. The prevalence of IRS was 11.25%. As expected, youth with IRS displayed significantly higher BMI, systolic and diastolic BP, TC, LDL-C, apoB, TG, FFA, insulin, and glucose as well as lower levels of HDL-C and apoA-I than youth without IRS. No differences were detected in age, sex, cigarette smoking, and alcohol intake between the two groups.

All 1,742 individuals were genotyped for the Ala54Thr polymorphism in the FABP2 gene and the G-493T polymorphism in the MTP gene. At both loci, the distribution of genotypes was not significantly different from that expected under the Hardy-Weinberg equilibrium ($P = 0.384$ and 0.198, respectively). There were no significant differences in FABP2 and MTP allele or genotype frequencies between subjects with and without IRS (**Table 2**). Similarly, there were no significant associations between FABP2 variants and IRS or between MTP variants and IRS (**Table 3**).

We examined the effect of FABP2 polymorphism on mean lipid, apoA-I, apoB, FFA, glucose, and insulin levels. Because we did not detect heterogeneity of effect of FABP2 polymorphism by sex or age [with the exception of insulin (for which the *P* value for the interaction FABP2 × sex = 0.014), all interactions *P* values were >0.1], sex and age groups were pooled in subsequent analyses. We observed a heterogeneity of FABP2 effect by IRS status on TC, LDL-C, TG, and insulin (*P* for interaction = 0.045, 0.018, 0.017, and 0.044, respectively). Therefore, subsequent analyses were conducted for each IRS subgroup separately (IRS− and IRS+). Mean concentrations of TC and LDL-C were lowest in IRS+ subjects with the FABP2 Ala/Ala genotype (**Table 4**; *P* for comparisons between Ala/Ala and Thr/Thr genotypes = 0.040 and 0.020 for TC and LDL-C, respectively). Although not statistically significant, a similar trend was observed for apoB. Mean concentrations of HDL-C, apoA-I, TG, FFA, glucose, and insulin were similar among IRS+ subjects with different FABP2 genotypes. In IRS− subjects, the mean apoA-I concentrations tended to be higher in Ala/Thr heterozygotes than in Ala/Ala or Thr/Thr homozygotes ($P = 0.098$ and 0.065, respectively); mean concentrations of all other biochemical variables were similar among IRS− subjects with different FABP2 genotypes.

Because IRS was defined as hyperinsulinemia in combination with two or more other variables (overweight, high systolic BP, IFG, high TG, and low HDL-C), we tested whether specific metabolic components of IRS could explain the heterogeneity of the FABP2 effect by IRS status on TC, LDL-C, and apoB. We did not detect significant interactions between the FABP2 polymorphism and BMI, insulin, glucose, and HDL-C on mean levels of TC, LDL-C, and apoB (all $P > 0.1$). However, there was heterogeneity of the FABP2 effect by plasma TG concentrations. **Table 5** shows regression coefficients for main effects and for interaction terms between *Z* scores for TG concentration and FABP2 genotype in determining TC, LDL-C, and apoB levels. Regression coefficients for the interaction effect in the Thr/Thr individuals were statistically significant for the three dependent variables compared with Ala/Ala subjects. **Figure 1** depicts the effect modification of the associations between TG and TC, LDL-C, and apoB by FABP2 genotype. The slopes of the regression lines for the Thr/Thr homozygotes were statistically significantly different from those for the Ala/Thr heterozygotes ($P < 0.001$ for TC, LDL-C, and apoB) and from those for the Ala/Ala homozygotes ($P < 0.001$ for TC, LDL-C, and apoB), whereas the slopes of the regression lines were sim-

TABLE 4. Biochemical characteristics of study participants by IRS status and FABP2 genotype

	IRS−				IRS+			
	FABP2 Genotype				FABP2 Genotype			
Variable	Ala/Ala (n = 851)	Ala/Thr (n = 582)	Thr/Thr (n = 113)	*P*[a]	Ala/Ala (n = 112)	Ala/Thr (n = 73)	Thr/Thr (n = 11)	*P*[a]
TC (mmol/l)	3.96 ± 0.76	3.98 ± 0.70	3.98 ± 0.86	0.816	4.07 ± 0.71	4.21 ± 0.90	4.69 ± 0.68	0.026
LDL-C (mmol/l)	2.28 ± 0.65	2.27 ± 0.59	2.35 ± 0.75	0.303	2.35 ± 0.58	2.55 ± 0.80	2.92 ± 0.48	0.006
ApoB (g/l)	0.65 ± 0.17	0.65 ± 0.16	0.67 ± 0.22	0.150	0.73 ± 0.18	0.74 ± 0.19	0.85 ± 0.18	0.107
HDL-C (mmol/l)	1.32 ± 0.25	1.34 ± 0.25	1.27 ± 0.21	0.066	1.09 ± .017	1.12 ± 0.20	1.10 ± 0.23	0.426
ApoA-I (g/l)	1.19 ± 0.17	1.22 ± 0.18	1.17 ± 0.15	0.021	1.11 ± 0.16	1.10 ± 0.16	1.11 ± 0.16	0.889
TG (mmol/l)[b]	0.80 ± 0.33	0.82 ± 0.36	0.81 ± 0.32	0.630	1.40 ± 0.70	1.18 ± 0.48	1.47 ± 0.96	0.123
FFA (mmol/l)[b]	0.43 ± 0.21	0.44 ± 0.21	0.42 ± 0.21	0.977	0.46 ± 0.19	0.42 ± 0.17	0.52 ± 0.25	0.085
Glucose (mmol/l)	5.14 ± 0.38	5.14 ± 0.36	5.18 ± 0.39	0.547	5.33 ± 0.41	5.33 ± 0.41	5.33 ± 0.38	0.775
Insulin (pmol/l)[b]	37.0 ± 17.3	38.1 ± 18.3	38.8 ± 21.0	0.360	97.7 ± 66.6	80.8 ± 33.3	83.2 ± 25.6	0.097

Data are expressed as means ± SD.
[a] *P* value for comparisons between genotypes after adjustment for school, age, sex, cigarette use, and alcohol intake.
[b] Untransformed data are presented; $\log_e$-transformed values were used for formal statistical comparisons.

TABLE 5. Multiple mixed regression analysis showing the interaction of FABP2 genotype with the effect of TG concentrations on TC, LDL-C, and apoB concentrations

	Dependent Variable					
	TC		LDL-C		ApoB	
Explanatory Variable	β (SEM)	*P*	β (SEM)	*P*	β (SEM)	*P*
	mmol/l		*mmol/l*		*g/l*	
Z score for TG (1 SD)	0.362 (0.020)	< 0.001	0.180 (0.020)	< 0.001	0.082 (0.005)	<0.001
FABP2 genotype						
Thr/Thr	0.119 (0.054)	0.029	0.124 (0.054)	0.021	0.034 (0.013)	0.012
Ala/Thr	−0.010 (0.029)	0.735	−0.006 (0.029)	0.833	−0.004 (0.007)	0.590
Interaction term						
Z score for TG × Thr/Thr	0.250 (0.058)	< 0.001	0.240 (0.057)	<0.001	0.059 (0.014)	<0.001
Z score for TG × Ala/Thr	−0.015 (0.029)	0.610	−0.009 (0.029)	0.762	−0.004 (0.007)	0.542

ilar for Ala/Thr heterozygotes and Ala/Ala homozygotes ($P = 0.588$, 0.737, and 0.554 for TC, LDL-C, and apoB, respectively). Similarly, the associations between FABP2 genotype and TC, LDL-C, and apoB were modified by plasma TG concentrations. As shown in **Fig. 2**, the Thr/Thr genotype was associated with a trend toward lower values for TC, LDL-C, and apoB compared with Ala/Thr or Ala/Ala genotypes in subjects with lower TG levels (age- and sex-specific *Z* score for TG < -1). Conversely, the Thr/Thr genotype was associated with a significant increase in TC ($P = 0.005$ and 0.006, respectively), LDL-C ($P = 0.009$ and 0.008, respectively), and apoB ($P = 0.004$ and 0.005, respectively) compared with Ala/Thr or Ala/Ala genotypes in subjects with higher TG levels (age- and sex-specific *Z* score for TG ≥ 1).

Finally, we studied the effect of the MTP polymorphism on mean lipid, apolipoproteins, FFA, glucose, and insulin levels. We did not detect a heterogeneity of MTP effect by sex, age, or IRS status (all $P > 0.05$). Therefore, all subjects were pooled for subsequent analyses. Mean concentrations of TC, LDL-C, apoB, HDL-C, apoA-I, TG, FFA, glucose, and insulin were similar among subjects with different MTP genotypes (**Table 6**).

DISCUSSION

An important new finding of this study is the demonstration of an interaction between FABP2 gene variants and IRS status in determining fasting plasma TC, LDL-C, and apoB concentrations. Moreover, among the metabolic components of IRS, only TG level displayed an interaction with the FABP2 polymorphism, which was stronger than the interaction between FABP2 and IRS. This result suggests that TG concentration is the metabolic component responsible for the heterogeneity of FABP2 effect by IRS status on TC, LDL-C, and apoB.

The polymorphism at codon 54 of the FABP2 gene could alter the functional properties of I-FABP, a protein expressed only in the absorptive enterocytes of small intestinal villi. The increased affinity of Thr-containing I-FABP for FA modifies intestinal fat absorption (14), postprandial lipid metabolism (13), and dyslipidemia (17, 30). Although several investigators have reported an association between the Ala54Thr polymorphism and insulin sensitivity in adults (19, 31, 32), others have not demonstrated the influence of FABP2 gene variants on insulin levels and insulin resistance (16–18). Our data revealed no significant

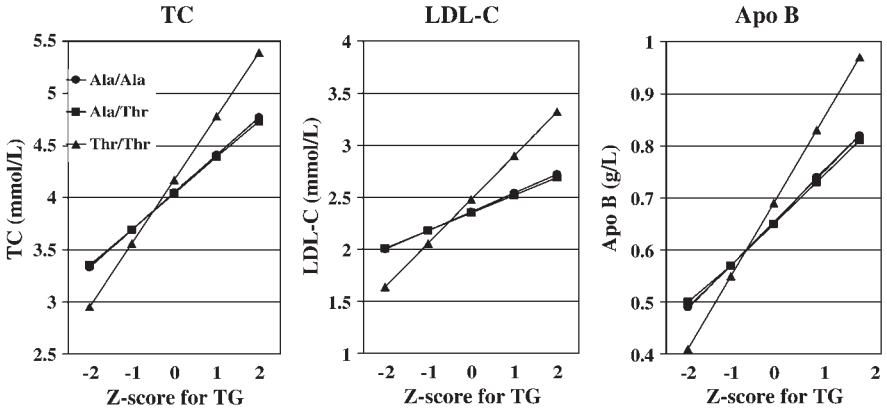

Fig. 1. Mean total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and apolipoprotein B (apoB) levels by triglyceride (TG) concentration and fatty acid binding protein-2 (FABP2) genotype. *Z* scores for TG concentration were estimated from the study distribution. Means were adjusted for school, age, sex, cigarette use, alcohol intake, apoE genotype, and *Z* scores for body mass index (BMI), insulin, and high density lipoprotein-cholesterol (HDL-C).

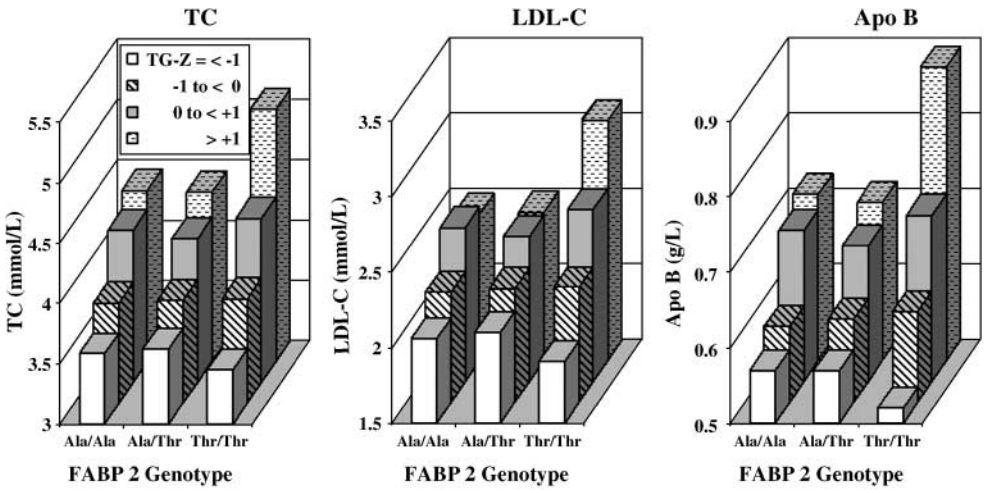

Fig. 2. Mean TC, LDL-C, and apoB levels by FABP2 genotype and TG concentration. TG categories were defined as follows: *Z* score for TG of <-1, *Z* score for TG of ≥ -1 and <0, *Z* score for TG of ≥ 0 and <1, and *Z* score for TG of ≥ 1. For the Ala/Ala genotype, the numbers of subjects within each TG category were 148, 373, 296, and 146 from the lowest (*Z* score for TG of <-1) to the highest (*Z* score for TG of ≥ 1) TG categories, respectively. For the Ala/Thr and Thr/Thr genotypes, the corresponding subject numbers were 100, 243, 213, 99 and 20, 40, 50, 14, respectively. Means were adjusted for school, age, sex, cigarette use, alcohol intake, apoE genotype, and *Z* scores for BMI, insulin, and HDL-C.

association between FABP2 polymorphism and fasting insulin. However, our study is among the few investigations conducted in a pediatric population. Because intracellular accumulation of TG in insulin-responsive tissues such as muscle and liver leads to insulin resistance (33), it is possible that the effect of the FABP2 polymorphism on fasting insulin or insulin resistance is modified by age. Longer exposure to the enhanced intestinal fat absorption associated with the Thr/Thr variant could lead to greater accumulation of TG in insulin-responsive tissues with age and a greater effect on insulin sensitivity. Further studies are required to clarify these hypotheses, especially because targeted gene disruption of FABP2 in mice did not compromise dietary fat absorption in vivo but was associated with the development of insulin resistance (34). It remains possible that other FABPs or lipid carriers are capable of compensating for the deficient function assumed by the disrupted gene (35, 36).

TABLE 6. Biochemical characteristics of study participants by MTP genotype

Variable	MTP Genotype			
	G/G (n = 936)	G/T (n = 696)	G/T (n = 110)	*P*[a]
TC (mmol/l)	3.99 ± 0.77	4.00 ± 0.75	3.98 ± 0.70	0.983
LDL-C (mmol/l)	2.31 ± 0.65	2.30 ± 0.64	2.31 ± 0.61	0.944
ApoB (g/l)	0.66 ± 0.18	0.66 ± 0.17	0.66 ± 0.18	0.981
HDL-C (mmol/l)	1.29 ± 0.25	1.30 ± 0.30	1.30 ± 0.24	0.805
ApoA-I (g/l)	1.19 ± 0.17	1.19 ± 0.18	1.19 ± 0.17	0.972
TG (mmol/l)[b]	0.86 ± 0.40	0.87 ± 0.46	0.83 ± 0.36	0.684
FFA (mmol/l)[b]	0.43 ± 0.21	0.43 ± 0.21	0.46 ± 0.22	0.300
Glucose (mmol/l)	5.16 ± 0.38	5.17 ± 0.38	5.17 ± 0.36	0.801
Insulin (pmol/l)[b]	43.6 ± 29.2	43.9 ± 32.6	40.0 ± 19.3	0.452

Data are expressed as means ± SD.

[a] *P* value for comparisons between genotypes after adjustment for school, age, sex, cigarette use, and alcohol intake.

[b] Untransformed data are presented; $\log_e$-transformed values were used for formal statistical comparisons.

The FABP2 Thr/Thr genotype was associated with a much steeper increase in TC, LDL-C, and apoB in parallel to TG concentrations than the Thr/Ala and Ala/Ala genotypes. As demonstrated in Fig. 1, the interaction between FABP2 and TG clearly suggested a recessive effect, because no gene-dose response was found: the regression slopes for the associations between TG and TC, LDL-C, and apoB were similar for Ala/Thr heterozygotes and Ala/Ala homozygotes.

As a consequence of the significant interaction between FABP2 genotype and TG concentrations, the Thr/Thr genotype is associated with a decrease in mean TC, LDL-C, and apoB concentrations compared with the Thr/Ala or Ala/Ala genotypes in subjects with lower TG concentrations and with an increase in mean TC, LDL-C, and apoB in individuals with higher TG concentrations. This gene-lipid interaction and the low prevalence of the Thr/Thr homozygotes could explain some of the discrepancies between studies, which reported variable effects of FABP2 genotype on lipids (17, 30, 37).

The mechanism explaining the association between the FABP2 Thr/Thr variant and a steeper increase in fasting TC, LDL-C, and apoB in conjunction with TG is unknown. In vitro experiments using Caco-2 cells that were modified genetically (38) and studies with human intestinal explants (14) demonstrated the efficiency of the FABP2 Thr/Thr variant to enhance intestinal fat absorption compared with the Ala/Ala variant. We speculate that high levels of postprandial FFA transported by chylomicrons and chylomicron remnants could result in increased hepatic synthesis of VLDLs that are, in turn, catabolized to LDL particles. This is consistent with the greater postprandial TG

response in Finns with increased circulating VLDLs (13) as well as with high plasma cholesterol concentrations accompanied by reduced excretion of fecal bile acids (39).

The Thr-encoding allele has been shown to be associated with a variety of phenotypes. According to our data, the effects of allelic variations of FABP2 on lipid traits are context dependent. Recently, Damcott et al. (40) reported that genetic variation in FABP2 promoters affects transcriptional activity and leads to alterations in body composition and lipid processing in Hispanic and non-Hispanic subgroups in the San Luis Valley Diabetes Study. Differences in transcriptional activity have been suggested to result from the FABP2p-ID haplotype. More recently, Formanack and Baier (41) have shown that genotypes of variations in the FABP2 promoter in Pima Indians were in complete concordance with Ala54Thr. As stated by these investigators, in vivo phenotypic associations previously attributed to the Ala54Thr substitution could instead be attributable to the variant promoter carried on the same allele. Therefore, it would be interesting to perform sequences analyses to establish whether French-Canadian children with homozygous Thr/Thr or Ala/Ala genotypes have the FABP2p-ID or the variant promoter described by the aforementioned investigators (40, 41).

An association has been reported between the G-493T polymorphism in the promoter of the MTP gene, on the one hand, and either a reduction in TC, LDL-C, and apoB or an increase in BMI, plasma insulin levels, and the secretion of TG-rich lipoproteins, on the other hand (42–44). In contrast, the G-493T polymorphism was associated with increased levels of TC, LDL-C, TG, and apoB in young African-American men from the Coronary Artery Risk Development in Young Adults Study (21), whereas no association between the G-493T polymorphism and lipid phenotype could be demonstrated in the Framingham Offspring Study cohort (45). In the present study, the G-493T polymorphism in the MTP gene promoter was not associated with IRS or with variations in lipid, lipoprotein, or glucose traits in French-Canadian youth. The relationship between insulin resistance and MTP gene variants warrants further investigation, given that the promoter region of the MTP gene contains a negative insulin response element and that insulin, acting through its receptor, can decrease MTP expression (46, 47).

Although we could not detect a relationship between FABP2 gene variants, the G-493T polymorphism, and IRS in our large sample of French-Canadian youth, our data indicate an interaction between the FABP2 polymorphism and IRS status in determining plasma concentrations of TC, LDL-C, and apoB. In particular, among the numerous metabolic components, TG displayed a robust association with the FABP2 polymorphism. Because these multiple clinical phenotypes strongly increase the risk for cardiovascular disease, special attention should be assigned to uncovering FABP2 Thr variants in IRS in French-Canadian children and adolescents. Furthermore, these adverse outcomes should be examined in youth from different origins and call for the development of a long-term follow-up study. Finally, the precise mechanisms underlying the impact of the FABP2 polymorphism on lipid abnormalities in IRS deserve additional studies.

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